MICRO-CHEMICAL STUDIES ON NORMAL CERUMEN

II. The Percentage of Lipid and Protein in Casual and Fresh Cerumen*

S. P. CHIANG, Ph.D., O. H. LOWRY, M.D. and B. H. SENTURIA, M.D.

(With technical assistance of E. S. Baumann and B. C. Adler, M.D.)

A knowledge of the composition of the surface film of the skin of the external auditory canal is of fundamental importance if we are to understand its possible protective function. Since the composition of casual† cerumen that has remained in the ear for a period of weeks or months may not be the same as that of fresh cerumen, the study herein reported was undertaken to measure any difference between the two.

Nakashima (1), in 1933, found 18 per cent lipid material in pooled samples of dried, casual cerumen, whereas Akobjanoff *et al.* (2) extracted 9.5 per cent lipid from undried, pooled samples of casual cerumen. Chiang, Lowry and Senturia (3) found the average lipid content in dried, individual casual samples of normal cerumen to be 59.0 per cent for 38 children and 48.9 per cent for 82 adults. The individual lipid values ranged from 6.4 to 88.9 per cent in the children, and from 17.6 to 79.4 per cent in the adults (4). The authors concluded that sex and age differences had no significant effects upon the concentrations of lipid in the cerumen of adults.

Kirk (6), however, who measured the secretion of lipids on the forehead, found a significant difference between the amounts (rather than percentages) of lipid secreted by old men and old women, but no differences in the younger groups. Herrmann *et al.* (7) reported that another factor, the delivery of sweat to the skin surface, influences the amount of lipids on the skin surface. They demonstrated a parallelism between increased sweating and increased recovery of ether-soluble substances, except in the axillary vaults.

In estimating the protein in cerumen, Nakashima (1) found 43 per cent in dried, pooled cerumen. Chiang *et al.* (3, 4) found an average of 16.6 per cent protein, with a range from 5.2 to 36.8 per cent, in the dried, casual cerumen of 38 children. The average for 82 adults was 23.8 per cent protein, with a range of 9.2 to 55.6 per cent.

EXPERIMENTAL

Subjects: All of the eleven healthy volunteer adults who served as subjects had grossly normal cerumen. Since these volunteers were not always available

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* From the Departments of Otolaryngology and Pharmacology, Washington University School of Medicine, St. Louis, Missouri.

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† The term casual cerumen is not to be confused with "casual level", which was introduced by Herrmann and Prose (5) to designate the amount of ether-soluble substances that were obtained from an area of the skin that had been neither protected nor cleaned in the preceding 24 hours.

simultaneously, it was not possible to establish a rigid routine regarding the periods between the collecting of samples.

Collection of Samples: Casual specimens were obtained with an ear curette from the surface of the skin of the cartilaginous meatus of the external ear canal, without previous cleansing or treatment. The samples were stored in a deep freeze in small, tightly covered, glass bottles.

After the removal of wax for casual specimens, the ears were thoroughly irrigated with tap water and wiped with cotton swabs which were moistened with an alcohol-ether (3:1) mixture. Specimens of fresh cerumen were collected 24 or 48 hours later, and at intervals thereafter, by wiping the canal with a small piece of weighed, fat-free cleansing tissue.

Methods: Fresh samples and representative portions of casual samples, which were also placed on weighed pieces of cleansing tissue, were dried, weighed, and analyzed for percentages of lipid and protein as previously reported (3). The lipid content was estimated by the loss in weight after extracting the samples three times with redistilled ethanol.* The protein was determined photometrically, using a phenol reagent according to the method of Lowry *et al.* (8). The remaining portion of the original cerumen which was accounted for neither as extracted lipid nor as protein was designated as the residue.†

RESULTS

Table I shows the per cent lipid and protein and the per cent residue (by difference), in casual specimens and in specimens of fresh cerumen which were obtained from 16 ears of 11 persons at various intervals. The per cent of lipid in casual samples averaged 36.8, with a range of 13 to 64. The average per cent of lipid in fresh samples varied from 67.0 on the first day, to 77.5 on the third day to 54.0 for all specimens obtained on the eleventh or the fourteenth day. Individually, all specimens of fresh cerumen contained much higher percentages of lipid than did the corresponding casual samples. In practically all persons, the per cent of lipid in the fresh cerumen rose sharply to a peak during the first few days after cleaning, but thereafter it apparently did not follow a very consistent pattern: in some subjects, the high proportion of lipid was maintained for 11 days or longer; but in others, the per cent lipid dropped abruptly after the peak was reached, and then leveled off. The average percentage of lipid in fresh cerumen showed a gradual decrease with time, but it remained significantly higher than the mean for casual specimens throughout the experimental period, as shown in the table.

The per cent of protein in casual samples averaged 26.4 with a range of 15 to 40. The average per cent of protein in fresh samples varied from 7.6 on the first day, to 15.1 on the third day, to 26.6 on the eleventh or fourteenth day. In 14 of the 16 individual ears, the first specimens of fresh cerumen contained

* Ethanol was found to be as efficient as ethyl ether in extracting lipids from the small quantities of cerumen used in this study.

† In a previous publication (3) this calculated residue was referred to as non-lipid non-protein.

TABLE I

Per cent lipid, protein and residue of casual and fresh cerumen

Subject	Ear	Age	Sex	Casual			Days after Cleaning																
							1			2			3			4			7			11		
				Lipid	Protein	Residue	L	P	R	L	P	R	L	P	R	L	P	R	L	P	R	L	P	R
A	R	27	M	18	33	49	60	9	31	76	15	9	79	12	9	75	15	10				46	28	26
	L			13	40	47	37	6	57	82	8	10	82	9	9	30	45	25				31	37	32
B	R	34	M	38	28	34	73	4	23	77	15	8				53	22	25				55	25	20
	L			36	29	35	46	5	49	41	10	49	65	25	10	40	40	20				43	33	24
C	R	27	M	58	20	22				85	14	1				74	15	11				53	11	36
	L			47	20	33				89	10	1				73	17	10				62	20	18
D	R	31	M	62	22	16	93	6	1				79	16	5	86	7	7				73	17	10
	L			54	28	18		10		81	11	8	81	14	5	74	14	12				69	21	10
E	R	49	F	49	28	23	84	15	1				83	10	7							67	19	14
	L			37	40	23	50	6	44				86	10	4							65	19	16
F	R	40+	F	15	27	58				51	27	22							41	32	27	45[c]	33[c]	22[c]
G	R	31	M	18	30	52				68	12	20				36	41	23	37	42	21	51[c]	38[c]	11[c]
H	L	46	M	64	15	21				74	11	15				75[b]	11[b]	14[b]						
J	L	39	M	33	27	40				78	16	6				74[b]	16[b]	10[b]	74					
K	L	31	M	33	24	43				72	15	13				76	11	13	68	13	19			
M	R	51	F	36	23	41				66	27	7												
Mean				36.8	26.4		67.0	7.6		71.6	15.5		77.5	15.1		64.2	20.8		55.0	29.0		54.0	26.6	
S.E.				5.1	1.8		9.5	1.2		3.5	2.0		4.3	3.5		5.9	4.1		9.4	8.5		4.1	3.3	
N[a]				11	11		4	4		10	10		4	4		8	8		4	3		7	7	

[a] The mean and standard error (S.E.) were computed by using the average value from both ears of one person (where these data were obtained) as a single datum.

[b] Specimens taken at 5 days instead of 4.

[c] Specimens taken at 14 days instead of 11.

lower percentages of protein than the casual samples, and in the other two ears there was approximately the same protein content in casual and fresh cerumen. In general, the percentages of protein in fresh cerumen increased rather quickly until they nearly equaled those in the casual specimens. The residue portion was much less in the fresh cerumen than in the casual.

DISCUSSION

It is suggested that the much higher lipid content in the specimens of fresh cerumen may be explained in the following manner. After thorough cleansing of the auditory canal, preformed sebum present in the follicular canal is extruded onto the surface of the skin, whereas exfoliation is not immediately increased. Thus, the highest lipid and lowest protein proportions occurred during the first few days after cleaning. In a few subjects, the maximum percentage of lipid was found on the first or fourth day after cleaning, but in most, the maximum occurred on the second or third day.

As the interval after cleansing increases, there is a restoration of the normal surface cover as the result of an increase in the accumulation of disintegrating epidermal cells and a relative reduction in the amount of sebum. Consequently, there is an increase in the percentage of protein (derived primarily from the keratin of the epidermal cells), and a decrease in the percentage of lipid. The lipid and protein proportions on the skin surface in the ear canal may therefore be considered to be dependent upon the relative rates of two processes: lipid delivery (9) and exfoliation.

Another factor should also be considered. Exfoliation might be increased above normal through possible irritation of the skin of the meatus by the daily collection of specimens. This irritation may produce an increased amount of protein in the samples, without a corresponding elevation in the amount of lipid recovered.

The residue portion was higher in specimens of casual cerumen than in fresh. It appears that this fraction is low immediately following cleansing, and slowly increases thereafter. The increase may be explained by the fact that some lipids (especially those containing unsaturated double bonds) may, upon contact with atmospheric oxygen, have undergone oxidation or other changes which rendered them unextractable with ethanol.

SUMMARY

In individual specimens of cerumen obtained from normal persons, there were large variations between the percentages of lipid, protein and residue. Nevertheless, there were substantial differences between the compositions of casual and fresh cerumen.

In fresh cerumen, the proportion of lipid was consistently higher than in casual specimens, whereas the proportion of protein was usually much less in fresh cerumen than in casual. With time, the average composition of fresh cerumen seemed to change until it approached that of the casual. This reversion toward the composition of casual cerumen was much more rapid with respect to protein than to lipid.

The differences between casual and fresh cerumen, and the changes which occur in the fresh secretion with time, were interpreted as reflecting a labile relationship between the delivery of lipids and the exfoliative processes occurring on the surface of the skin. Chemical changes in the lipid portion probably occur after extrusion from the follicular canal and also influence the percentage composition of cerumen.

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APPENDIX: STATISTICAL ANALYSIS

To examine the data statistically, only those data which could be handled in a uniform and consistent manner were selected from Table I. For all subjects in whom specimens had been taken from both ears, the data from only one ear (selected randomly) of each were used in the analyses.

The components of the casual cerumen were compared to the first fresh sample of cerumen and, in a separate analysis, to the last sample of cerumen. The component percentages were first transformed by use of the appropriate angular transformation (10). In each case then, the analysis of variance technique for factorial designs was used to analyze these data. The design included *subjects*, *casual* vs. *fresh cerumen*, and *lipid* vs. *protein* as "fixed" main effects and the interactions among these main effects (11). The second order interaction was used as the error term and the 5 per cent point as the critical point in these analyses. The results of these analyses are as follows:

(1) No difference was found between percentages of lipid and protein in the casual cerumen.

(2) There is a significantly greater proportion of lipid in fresh cerumen than in casual cerumen.

(3) There is a significantly smaller proportion of protein in fresh cerumen than in casual cerumen.

(4) When no specimen is taken between the 4th and 11th day, the proportion of lipid at the 11th day is still greater than in the casual, but the proportion of protein is not different from that in the casual cerumen.

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